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14. ABSTRACT

Development of targeted MDS therapies has been challenged by a limited insight of molecular mechanisms of disease pathogenesis and therefore, current therapeutic approaches remain highly empiric largely ignoring the role of the inflammatory bone marrow (BM) microenvironment. We identified S100A9 as a mediator of myeloid derived suppressor cell (MDSC) activation and as the native ligand to CD33, which is highly expressed in MDS. Therefore, our goals are to develop novel therapeutic strategies that inactivate MDSC to improve survival of HSPC and allow us to provide the basis for more effective, biologically rational MDS therapeutics. Based on our initial hypothesis of hematopoietic improvement through the targeting of the S100A9-CD33 pathway, this funding year we were able show that our proposed approaches can inactivate MDSC in vitro. This is achieved through the development of a specific S100A9 trap, the CD33-chimeric human IgG1 molecule (CD33-fusion). We demonstrated that CD33-fusion is able to neutralize soluble S100A9 and suppresses S100A9 induced pro-inflammatory cytokine production as well as improve hematopoiesis.

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1. INTRODUCTION

Understanding the selective pressures and mechanisms involved in the initiation of stem cell malignancies is critical for development of effective strategies for prevention and treatment. Myelodysplastic syndromes (MDS) are hematologically diverse bone marrow (BM) failure syndromes that share features of cytological dysplasia, ineffective hematopoiesis and a propensity for progression to acute myeloid leukemia (AML). MDS are senescence-dependent myeloid malignancies with a rising prevalence owing to the aging of the American population. Effective disease-altering therapies for patients with MDS are limited due largely to inadequate understanding of the precise pathobiological mechanisms involved in disease initiation and progression. Although innumerable somatic genetic events have been annotated in recent years, many of which are sufficient for disease initiation in murine models, microenvironmental factors conducive for emergence of these genetic events remain to be delineated. In the original proposal we hypothesized that inflammation and aging induce the accumulation of **myeloid-derived suppressor cells (MDSCs),** a heterogeneous group of immature myeloid cells, which play a critical role in MDS pathogenesis. A key finding of our recent studies was the identification of CD33 as a receptor that binds S100A9 to drive expansion and activation of BM MDSCs, and trigger cell death of myeloid progenitors. MDSC-mediated suppressive activity is stimulated by the danger-associated molecular pattern (DAMP) heterodimer S100A9, that heterodimerizes with its partner S100A8, and interacts with innate immune receptors involved in MDSC activation. Therefore **S100A9 is a key factor in MDSC activation.** Based on this preliminary evidence, we have hypothesized that CD33-S100A9 signaling is a critical driver of MDSC activation and MDS pathogenesis that can be therapeutically exploited in a selective fashion. We propose that inactivation of MDSCs through selective interference with S100A9/CD33 signaling offers a novel strategy for MDS therapeutic development. This approach would also further enhance our understanding of mechanisms underlying disease initiation. In the past year, we have made significant research progress toward this goal through the development of novel CD33-chimeric protein (CD33-fusion) to characterize its biochemical and biological activity.

2. KEYWORDS

Myeloid-derived suppressor cells (MDSC); Myelodysplastic syndromes (MDS); S100A9 proteins; inflammation, Genomic instability, bone marrow failure, hematopoiesis; Interleukin 10 (IL10); hematopoietic stem and progenitor cells (HSPC)

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals of the project are:

Aim 1: To determine if strategies to block S100A9/CD33 signaling in MDSC improves the BM microenvironment and rescues hematopoiesis. We are investigating our recently developed CD33-IgG1 chimeric receptor as an S100A9 ligand trap to neutralize S100A9 and thereby suppress MDSC activation.

Aim 2: To validate a novel MDSC-inactivating compound that disrupts CD33-ITIM signaling. We will interrogate our recently described S100A9-CD33 pathway to characterize a novel compound, ICTA, to inactivate inflammatory suppression through the maturation of MDSCs as a novel preclinical approach.

Goals for the first 12 months: Following the major objectives of the proposal we have set up two subtasks for the first 12 months: 1) To determine the neutralization efficiency of CD33-fusion using BM plasma of MDS patients; 2) To determine if the CD33-fusion can suppress CD33-ITIM signaling.

What was accomplished under these goals?

Under these goals, and following the SOW, the major research activities and accomplishments for the past funding period were:

1. We have tested the biochemical properties of the CD33-fusion protein. Based on the well-established studies that S100A9 is an intracellular molecule released by myeloid and tumor cells in response to inflammation, cell damage or infection and it is a critical driving factor for MDS pathogenesis, we attempted to find a novel therapeutic approach to suppress S100A9 mediated activity. Our major efforts and activities focused on how to characterize the CD33-IgG1 fusion protein that can compete for binding to S100A9 with surface CD33 receptor in vitro and determine the biological effect of CD33-fusion proteins.

(1) **SPR experiment shows that CD33-fusion directly binds recombinant human S100A9 (rhS100A9) in vitro.** To test the CD33 fusion protein binding ability and neutralizing capacity against S100A9, we performed *in vitro* studies of CD33-fusion binding affinity and specificity with rhS100A9 using Surface Plasmon Resonance (Biacore™) analysis. Either rhS100A9 was captured on a sensor chip with CD33-fusion injected over the captured protein, or vice versa. As shown in Figure 1, the SPR analysis demonstrate that CD33-fusion displays dose-dependent, high affinity binding with S100A9 confirming the reciprocal set up.

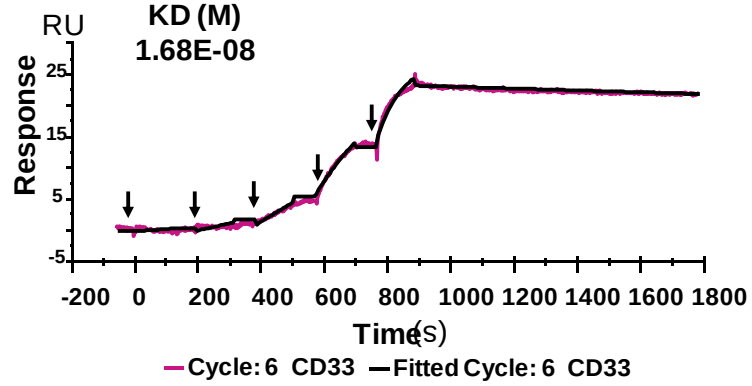

Figure 1. SPR analysis of CD-33-fusion. The kinetic fitting parameters are significant for the experimental data (standard errors (SE) are substantially less than 10% of the fitted value). The Biacore Dissociation constant (*Kd*) for CD33 binding to S100A9 = 1.68 x 10^{-8}

(2) **CD33-fusion can inhibit S100A9 induced inflammatory cytokine production in human mononuclear cells from healthy donors.** For the next task we investigated if CD33-fusion will have any biological effect in terms of inhibition of MDSC associated pro-inflammatory cytokine production. The buffy coats purchased from Florida Blood Services blood bank were used to isolate peripheral blood mononuclear cells from healthy donors (PBMC). The monocytes isolated from PBMC were treated or untreated with rhS100A9 at a concentration of 10 ug/ml for 48 hours. Both IL-10 secretion and gene expression (key readouts of S100A9/CD33 activation) were monitored using ELISA or real-time PCR (QPCR), respectively. As shown in Figure 2**,** the CD33-fusion displayed a significant inhibitory effect on IL-10

generation at both protein and gene expression levels. IL-10 is the major biological mediator produced by activated MDSCs via S100A9 stimulation and plays a critical role in a feed-forward model to activate MDSC. Our results indicate the potential therapeutic role of CD33-fusion in its role as an S100A9 trap.

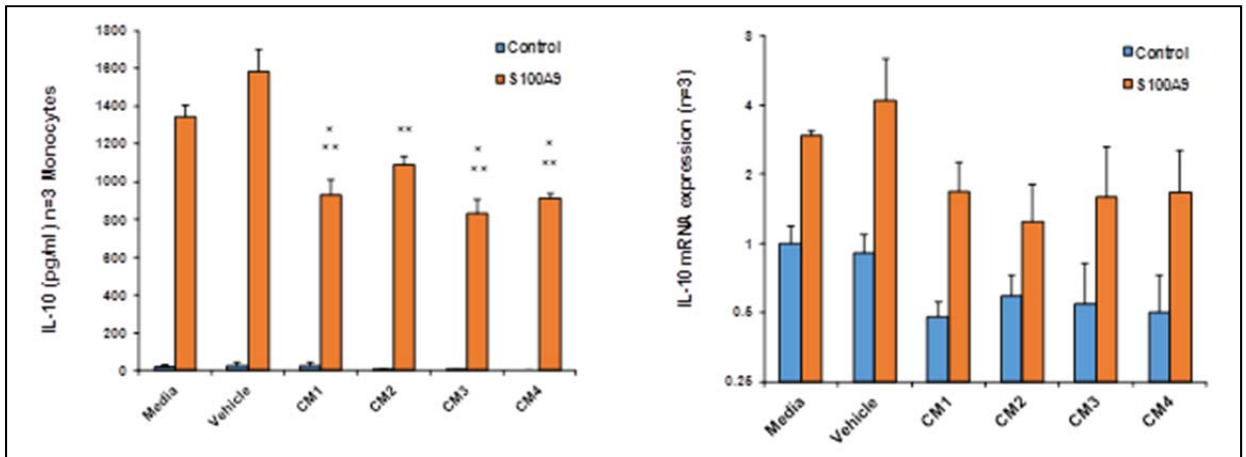

Figure 2.- The effect of CD33-fusion on IL-10 production in stimulated monocytes at the level of protein and gene expression.

(3) **CD33-fusion can neutralize S100A9 in bone marrow plasma to neutralize S100A9 mediated hematopoietic suppression.** Our preliminary investigation has shown that BM plasma from patients with MDS has a high level of S100A9. In general, the proinflammtory cytokines and soluble inflammatory mediators such as IL-10 generated by MDSC upon engagement with S100A9 are released in the local bone marrow microenvironment. All these factors associated with MDSCs activation act as a suppressive mediator and have the ability to damage hematopoietic progenitor and stem cells (HPSC), reflecting hematopietic suppression (anemia), a common feature seen in patients with MDS. Our major hypothesis is that S100A9 is the key initiator in the activation of MDSC in a feed-forward manner that can inhibit HSPC function. To validate our hypothesis by targeting S100A9 via neutralization of S100A9 in the bone marrow to improve hematopiesis, we pretreat bone marrow

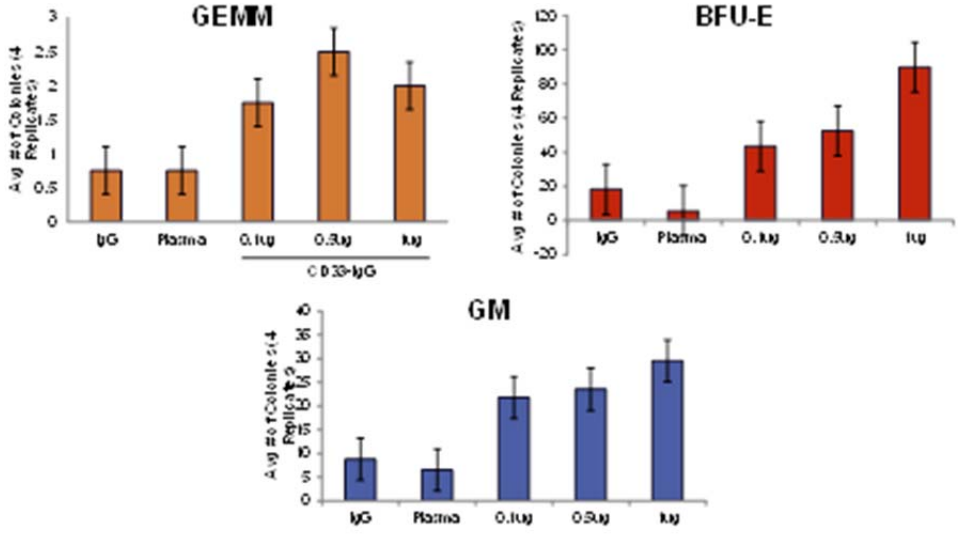

Figure 3.- Neutralization of Plasma S100A9 by CD33 Chimera Trap Enhances Colony Forming Capacity in MDS Patient Specimens

plasma from MDS patients with or without CD33-fusion and re-culture it with HSPC to check their proliferation using a colony formation assay. As shown in a representitative experiment in Figure 3, treatment with CD33-fusion greatly improved colony formation when compared with control treated plasma. These results are key finding indicating that CD33-fusion has the potential to improve hematopoietic suppression and BM failure syndrome.

(4) **Create a Tetra-chimera to enhance S100A9 binding.** Following our original proposal/SOW in order to achieve the projected goal of enhancing the effectiveness of the CD33-fusion, increase its binding capacity and improve ligand neutralization in preparation for future clinical trials, we have engineered a tetra-globular molecule by taking advantage of both the variable region of CD33 and the variable region of another S100A9 binding receptor, RAGE, to create an enhanced S100A9 trap with higher binding efficiency. This design is based on the well-established study showing that RAGE can bind to S100A8/A9 heterodimer with a different conformation than CD33. This work is still in the design and construction phase which is the main reason why we have not generated any significant data yet. While the complexity of the molecular design was challenging and large amounts of labor were required we have been able to produce some of these variants that we should be able to test within the next funding period.

In summary, we have made significant research progress under the proposed goals, in line with the SOW, for the first 12 months.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

We will follow our original proposal and SOW to further investigate our novel therapeutic approaches targeting S100A9 activated MDSC. 1) In addition to testing the CD33-fusion, we will expand our strategies to use human engineered anti-CD33 antibody to test its effect on antibody dependent cytotoxicity (ADCC); 2) We will test the novel MDSC inactivating compound ICTA, developed in our lab, in S100A9 transgenic mice. This will allow us to confirm and validate our novel approaches in an S100A9-associated disease model.

4. **IMPACT:**

MDS are the most common bone marrow failure (BMF) with variable survival ranging from a median of months in patients with high risk disease to years in low-risk patients. More than 50,000 cases are diagnosed annually in the US with the overall disease burden increasing as a result of the aging of the population in a disease characterized by high morbidity and mortality. Currently available treatment options have limited effect and can be associated with severe side-effects and high economical costs. This is mainly due to previous studies of the molecular

pathogenesis of MDS focusing primarily on the already genetically altered malignant HSPC responsible for propagation of the malignant clone, rather than selection pressures conducive to the emergence of the clone. As a consequence, current treatments are largely empiric and non-specific. Instead, our hypothesis focuses on targeting S100A9/CD33 pathways to block the activation and accumulation of MDSCs in the local bone marrow microenvironment that suppresses healthy HSPC and induce DNA instability promoting MDS clone expansion. Our major findings obtained in the past year demonstrated that CD-33-fusion has a potential as a therapeutic approach to target MDSC activation and feasibly address a conceptually-novel hypothesis.

What was the impact on the development of the principal discipline(s) of the project?

We propose a paradigm shift that stems from a concept that understanding how the inflammatory microenvironment contributes to HSPC damage and malignant evaluation will have a profound and far-reaching effect on understanding MDS pathophysiology. If successful, this combined in vitro/in vivo approach can lead to immediate clinical application. Therefore, the conditions that these therapeutic approaches address represents an unmet medical need for a disease with few available treatments, thus providing a unique opportunity for success through the development of effective targeted therapeutics, like ours, in this patient population where it would have enormous clinical impact.

What was the impact on other disciplines? Nothing to Report

What was the impact on technology transfer? Nothing to Report

What was the impact on society beyond science and technology? Nothing to Report

5. CHANGES/PROBLEMS: Nothing to Report

6. **PRODUCTS:** Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS: None

What individuals have worked on the project?

Name:	Sheng Wei
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Dr. Wei is the PI of this project and for the overall direction of proposed project and He will oversee all molecular validation

	studies and data analysis and interpretation of experimental results.
Funding Support:	No change

Name:	Xianghong Chen
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	She is responsible for carring out all the biological and biochemical assays and make all the novel constructs
Funding Support:	No change

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to Report

- **What other organizations were involved as partners?** Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: None